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The Enhancement of Growth and Feeding Performance of Persian Sturgeon (*Acipenser persicus*) Larvae by *Artemia urmiana* Nauplii Bioencapsulated via Baker's Yeast (*Saccharomyces cerevisiae*)

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Abstract: In this study *Artemia urmiana* was used as a vector to carry probiotic Baker's yeast (*Saccharomyces cerevisiae*) to digestive tract of Persian Sturgeon (*Acipenser persicus*) larvae. The yeast (*Saccharomyces cerevisiae*) under the commercial title of Thepax was prepared from Doxal Co., Italy and was used for bioencapsulation of *Artemia urmiana*. This experiment was conducted in a completely random design. *Artemia urmiana* with three concentrations of Baker's yeast, 5, 5.30 and 5.48 Log CFU mL^{-1} in suspension of broth for 10 h was bioencapsulated and was fed by Persian Sturgeon larvae. The Persian Sturgeon larvae were fed on the base of the 30% of their body weight for six times a day. The control treatment were fed on unbioencapsulated *Artemia urmiana*. The results indicated that the *Saccharomyces cerevisiae* could influence growth and feeding parameters in Persian Sturgeon larvae. The final body weight and Specific Growth Rate (SGR) in experimental treatments had significant difference in comparison with control treatment ($p<0.05$). Food Convertion Efficiency (FCE) was increased significantly. The Baker's yeast had significant positive effects on Conversion Efficiency Ratio (CER), Thermal Growth Coefficient (TGC), Velocity of growth body Weight (VW %) and Velocity of growth body Length (VL %) in comparison with control treatment ($p<0.05$). Also the Relative Food Intake (RFI) significantly decreased ($p<0.05$) while crude protein and energy significantly increased ($p<0.05$). The maximum of Lpid Productive Value (LPV) and Protein Productive Value (PPV) were obtained in treatment of T2 ($p<0.05$). This study showed that *S. cerevisiae* had high efficiency in feeding parameters and growth performance of Acipenser persicus larvae.

Key words: Bioencapsulation, *Artemia urmiana*, Baker's yeast, protein gain, lipid productive value, Iran

INTRODUCTION

Probiotics can be defined as live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance (Fuller, 1989). Most studies on the effects of probiotics on cultured aquatic animals have emphasized a reduction in mortality or the improved resistance against putative pathogens (Irianto and Austin, 2002; Deeseenthum *et al*., 2007). However, the beneficial effects are sometimes temporal, depending on the time of exposure (Verschuere *et al*., 2000).

The *Artemia urmiana* is common live food organisms used for the rearing of marine fish larvae (Fazeli and Azari-Takami, 2006; Hafezieh *et al*., 2008; Fazeli *et al*., 2008). These have been considered as possible vectors for the delivery of different substances such as nutrients and probiotics. Intensive rearing of marine fish larvae suffers from heavy mortalities which may be attributed to bacteria introduced in the rearing system with live food (Keskin and Rosenthal, 1994). Optimization of microbial compositions and load in live food during the process of bioencapsulation is one of the most important concerns in aquaculture as it can reduce the heavy mortalities which often occur during the rearing of fish larvae (Olsen, 1997). In the last decade, the scientific community carefully examined roles and effects of probiotics in aquaculture as an alternative to antimicrobial drugs, demonstrating positive effects on fish survival (Villamil *et al*., 2002), growth (Burr *et al*., 2005), stress resistance (Smith and Davey, 1993; Rollo *et al*., 2006), immunosystem enhancement (Erickson and Hubbard, 2000; Picchietti *et al*., 2007) and finally general welfare (Balcazar *et al*., 2006; Silvi *et al*., 2008).

The use of natural prophylactic supplements in place of chemotherapeutics in aquaculture has received a great

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deal of attention in the past decade such preventive products include probiotics. These biotics can be applied through external bathing or dietary supplementation and have been demonstrated to improve growth performance, feed utilization, digestibility of dietary ingredients, disease resistance and stimulate the immune response of aquatic animals (Gatesoupe, 2008; Kesarcodi-Watson *et al*., 2008; Wang *et al*., 2008; Merrifield *et al*., 2010).

The use of probiotic bacteria has been suggested as an important strategy to accomplish reproducible outputs through biocontrol in cultivation systems for marine fish larvae and crustaceans (Nogami and Maeda, 1992). The bacterial flora in the larval gut originates from the bacteria associated with the eggs, the water in the rearing tanks and the live food (Olafsen and Hansen, 1992). The gut of marine fish larvae is rapidly colonized by bacteria during the 1st days after hatching. Members of this pioneer community that colonize the gut at an early stage may acquire a competitive advantage compared with bacteria introduced at a later stage (Hansen and Olafsen, 1999). Successful colonization in digestive system of larvae involves competition with the established microflora for attachment sites and nutrients. The species composition of the intestinal microflora of fish larvae can be influenced at an early stage of development when few if any, bacteria are present in the larval gut by addition of specific bacterial strains to the live food or the water (Ringo *et al*., 1996).

Several studies demonstrated these positive effects using a single or two probiotic strains and just few studies described the effects of a mixture of probiotics in fish and shrimp aquaculture (Balcazar, 2003). Concurrently, *Bacillus* species can be found in marine environment and are part of the microflora of several marine species (Hovda *et al*., 2007). Little studies had been carried out to incorporate probiotics into a freahwater species common carp, *C. carpio* (Wang and Xu, 2006) and crustaceans, Indian white shrimp *Fenneropenaeus indicus* (Ziaei-Nejad *et al*., 2006) and shrimp *Penaeus vannamei*, (Wang, 2007) based on growth performances and digestive enzyme activities.

Live food (*Artemia urmiana*) have been used as vectors for delivering compounds of diverse nutritional and/or therapeutic value to larval stages of aquatic animals (Cappellaro *et al*., 1993), a process known as bioencapsulation. *Artemia urmiana* are able to graze bacteria (Michels and Mesters, 1998). The number of bacteria accumulated in live food during bioencapsulation depends on the concentration of the bacterial suspension and the bacterial strain applied (Gomez-Gill *et al*., 1998; Makridis *et al*., 2000). *Bacillus* sp. has been used as a probiotic and diet additive for various animals. It has been observed to be capable of enhancing growth parameters (Lara-Flores *et al*., 2003) of various fish species and thus, may serve as an excellent health promoter for fish culture. present study was conducted to evaluate the potential of the effects of different levels of the beneficial probiotic Baker's yeast (*Saccharomyces cerevisiae*) in bioencapsulation of *A. urmiana* on the exploitation of nutrient composition of this live food by Persian sturgeon (*Acipenser persicus*) larvae for promotion feeding parameters.

MATERIALS AND METHODS

About 10 days old Persian sturgeon (*Acipenser persicus*) larvae with initial weight of 50±7 mg and total length of 22±5 mm were obtained from Hatchery of Marjanii Sturgeon Center, Golestan, Iran. The Baker's yeast (*Saccharomyces cerevisiae*) under the commercial title of Thepax (contain 1×10^{10} cells mg^{-1}) was prepared from Doxal Co., Italy. The cysts of *A. urmiana* from the center of Artemia and Aquatic Animals in Urmia (Iran) were used for this study. The corions of the cysts were removed chemically by using the methodology that proposed by Sorgeloos *et al*. (1977). This process is known as decapsulation. Hatching of the decapsulated cysts was performed in glass cone with 1 L of seawater (3.0% salinity) at a density of 5.0 g L^{-1} and incubated at 30°C with constant illumination and aeration through setting air pump (Gomez-Gil *et al*. 1998). The bioencapsulation of *Artemia* nauplii were accomplished with density of 2 g live nauplii L^{-1} for 10 h and with three concentration of Baker's yeast, 5, 5.30 and 5.48 Log CFU mL^{-1} in suspension of broth for 10 h was bioencapsulated and was fed by Persian Sturgeon larvae. Three concentrations of yeast suspension (5, 5.30 and 5.48 Log CFU mL^{-1} in suspension of broth) were provided. Twelve fiberglass tanks (capacity of 50 L) with three replicates for experimental treatment and control were used. In experimental treatments of T1, T2 and T3 the Persian sturgeon larvae were fed by bioencapsulated *Artemia urmiana* by 5, 5.30 and 5.48 Log CFU mL^{-1} of suspension of broth, respectively. In control, the fish larvae were fed on unbioencapsulated *Artemia urmiana*. Each treatment was in triplicate. Healthy larvae of Persian sturgeon provided by the Fish Hatchery of Sturgeon Center of Marjanii. The fish were transferred to twelve 50 L circular fiberglass tanks. The density of fish larvae in per tank was 4-5 fish L^{-1}. Initial weight of fish larvae was 50 mg and total length 22 mm. Sturgeon larvae were fed based on the 30% of their body weight for six times a day at 2.00, 6:00, 10:00, 14:00, 18:00 and 22:00 with bioencapsulated *Artemia urmiana* in experimental treatments and unbioencapsulated *Artemia urmiana* in

control treatment, respectively. Each rearing tank was supplied with running fresh water which had been filtered through the special cotton filter (flow rate: 1 L min^{-1}). Water quality parameters from every tank were monitored each week throughout the experimental. The water temperature was 16.8±0.6°C, pH was 7.6-8.3 and water oxygen level was maintained above 7.5 mg L^{-1} during the experiment by setting electrical air pump. In the termination of experiment, fifty larvae from each tank were sampled and the total weight and length of body were measured.

Proximate composition of *Artemia urmiana*, fish carcass (initial and final of experiment) were analysed using the standard procedures described by the Association of Official Analytical Chemists (AOAC, 1990), moisture was determined by oven drying the weighed fresh sample at 100°C for 24 h; crude protein (nitrogen 6.25) by Micro-Kjeldahl digestion and distillation after acid digestion using a Kjeltec 1026 Distillation Unit together with a Tecator Digestion System (Tecator, Sweden), lipid was determined by extracting the residue with 40-60°C petroleum ether for 7-8 h in a Soxhlet apparatus and ash was determined by ignition at 550°C in a muffle furnace to constant weight.

Twenty fish from each tank were sampled at the termination of the feeding experiment, homogenized and analysed for moisture, crude protein, crude lipid and ash (on wet weight basis) following the aforementioned methods.

Some growth and feeding parameters of the fish were calculated based on the data of carcass analysis and biometry of *Acipenser persicus* larvae. The growth parameters and feeding parameters of the studied fish were calculated on the data of carcass analysis.

Results analyzed by one-way ANOVA and significant differents determined by Duncan test. All statistical was performed using the software SPSS 15.0 for Windows.

RESULTS

The growth parameters of Persian Sturgeon larvae in experimental treatments and control were shown in Table 1. Final Body Weight (FBW) in experimental treatments of larvae had significant difference in comparison with control treatment ($p<0.05$). The highest FBW (487.22 mg) was obtained in experimental treatment of T2. The *Saccharomyces cerevisiae* had significant positive effects on the Specific Growth Rate (SGR) and Thermal Growth Coefficient (TGC) in comparison with control treatment ($p<0.05$). The maximum of SGR (11.642% BW day^{-1}) and TGC (1.583%) were obtained in treatment of T2.

The growth parameter of Growth Coefficient Efficiency (GCE %) had the highest level (14.12%) in treatment of T2 and the treatment of T2 and T3 had significant difference with control treatment ($p<0.05$). The *Saccharomyces cerevisiae* in experimental treatments where the Persian Sturgeon larvae were fed by bioencapsulated *Artemia urmiana* with this probiotic yeast, significantly ($p<0.05$) increased the Velocity of growth body Weight (VW %) and Velocity of growth body Length (VL %). The best VW (11.405%) and VL (4.594%) were showed in T2 and T3, respectively. The survival rate was significantly ($p<0.05$) increased in T1 (89.00%) and T2 (92.00%) in comparison with control treatment (88.33%) but decreased in T3 (80.03%).

Proximate analysis of whole body of Persian sturgeon at the end of the feeding trial and mean values of feeding parameters were shown in Table 2 and 3. The best results in this trial obtained in experimental treatment where Persian sturgeon larvae were fed with bioencapsulated *Artemia urmiana*. The maximum of drymatter (12.01%) and minimum of mouisture (88.00%) were showed in treatment of T2.

The results indicated that the yeast of *Saccharomyces cerevisiae* significantly promoted leveles

Table 1: The growth parameters of Persian sturgeon larvae in different treatments

Parameters/Treatments	Control	T1	T2	T3
FBW(mg)	389.780±54.35^{b}	400.89±75.870^{a}	487.220±69.40^{a}	427.560±75.48^{a}
SGR (BW day^{-1} %)	10.046±2.342^{c}	10.284±2.173^{bc}	11.642±2.265^{a}	10.865±1.694^{b}
TGC (%)	1.367±0.294^{c}	1.397±0.281^{bc}	1.583±0.315^{a}	1.468±0.227^{b}
GCE (%)	9.430±2.860^{c}	9.810±1.090^{c}	14.120±2.530^{a}	11.090±2.290^{b}
VW (%)	10.768±1.100^{b}	10.888±0.955^{b}	11.405±0.843 ^{a}	11.160±0.667^{a}
VL (%)	4.037±0.951^{c}	4.293±0.708^{b}	4.555±0.674^{a}	4.594±0.573^{a}
Survival	88.330±1.530^{b}	89.000±1.730^{ab}	92.000±2.000^{a}	80.030±1.000^{c}

Table 2:Proximate composition of Persian sturgeon larvae (dry matter base) in feeding treatments

Parameters/Treatments	Control	T1	T2	T3
Dry matter (%)	9.67±0.8800^{b}	9.30±1.510^{b}	12.01±1.2500^{a}	8.75±0.5300^{b}
Mouisture (%)	90.33±0.8600^{a}	90.70±0.860^{a}	88.00±1.2700^{b}	91.30±0.5100^{a}
Crude protein (%)	77.08±1.3600^{a}	77.01±2.520^{a}	77.11±0.8900^{a}	76.97±1.4000^{a}
Crude lipid (%)	5.84±0.3000^{b}	6.76±0.500^{a}	6.74±0.5000^{a}	6.77±0.4000^{a}
Crude energy (kcal g^{-1})	4498.47±101.22^{b}	4798.23±99.01^{a}	4736.59±102.00^{a}	4780.45±180.20^{a}
Ash (%)	10.80±1.0000^{a}	10.65±1.500^{a}	10.42±1.9200^{a}	10.40±0.9500^{a}

Table 3: Mean values of some of feeding parameters in *Acipenser persicus* larvae

Parameters/Treatments	Control	T1	T2	T3
FCR	7.80±2.92000^{a}	7.52±2.49000^{a}	6.10±2.0300^{b}	6.65±1.6100^{b}
PPV	0.45±0.13000^{b}	0.46±0.12000^{b}	0.71±0.2200^{a}	0.46±0.1100^{b}
LPV	0.056±0.0170^{d}	0.064±0.0180^{c}	0.102±0.032^{a}	0.072±0.017^{b}
EPV	0.224±0.0410^{b}	0.237±0.0520^{b}	0.367±0.075^{a}	0.237±0.048^{b}
PER	6.39±1.89000^{c}	6.52±1.88000^{bc}	8.92±2.8000^{a}	7.12±1.6700^{b}
LER	10.82±3.2100^{c}	11.04±3.2100^{bc}	13.72±4.310^{a}	12.04±2.820^{b}
GSI (%)	18.30±3.2500^{b}	18.63±2.5800^{ab}	24.15±3.430^{a}	18.50±2.940^{b}
RFI (%)	30.67±133.82^{a}	20.41±127.10^{a}	25.99±99.88^{b}	27.85±99.85^{b}
FCE (%)	14.28±2.2300^{c}	14.57±1.2400^{bc}	18.11±3.690^{a}	15.90±2.720^{b}

of crude lipid, crude energy and carcass dry matter in experimental treatments in comparison with control treatment ($p<0.05$). While the crude protein and ash weren't significantly increased ($p>0.05$). The level of Crude lipid had significant difference in experimental treatments in comparison with control ($p<0.05$). The lowest (5.84%) and highest (6.77%) of crude lipid were obtained in control and treatment of T3.

In experimental treatments the Food Conversion Efficiency (FCE) increased while Food Conversion Ratio (FCR) and Relative Food Intake (RFI) decreased in comparison with control treatment ($p<0.05$).

The FCR of diets showed an inverse correlation with concentration of yeast (CFU L^{-1}) of bioencapsulated suspension of broth. Protein Efficiency Ratio (PER), Lipid Efficiency Ratio (LER) in treatments of T2 and T3 had significant difference in comparison with control treatment ($p<0.05$). The results indicated that the *S. cerevisiae* significantly enhanced leveles of Protein Productive Value (PPV), Lipid Productive Value (LPV) and Energy Productive Value (EPV) in experimental treatments in comparison with control ($p<0.05$). The highest of PPV (0.71), LPV (0.102) and EPV (0.367) were significantly higher than control ($p<0.05$). The Gastero Somatic Index (GSI %) in experimental treatment of T2 had significant difference with control ($p<0.05$).

DISCUSSION

The incorporation of probiotics via live food constitutes a very important potential tool for supplying probionts to the larvae. *Artemia urmiana* is one of the most important live foods that were used as a vector to carry yeast to digestive system of *A. persicus* larvae while the most studies about the probiotics concered with bioencapsulations by Artemia and rotifer. This study highlights the effects of yeast *S. cerevisiae* on the inhancement of growth of *A. persicus* larvae. The beneficial influence of *Saccharomyces cerevisiae* on growth parameters of *Acipenser persicus* larvae were completely observed. All the probiotic treatments of T1 and T2 resulted in growth and feeding performances better than that of control treatment while the treatment of T3 had lower than contol. The best performance of fish in terms of growth performance and feed utilization efficiency was recorded at the enrichment *A. urmiana* with 5.30 Log CFU of yeast L^{-1}. Then, this concentration of yeast was recognized the best level for process of bioencapsulations of *A. urmiana* by *Saccharomyces cerevisiae* in feeding of *Acipenser persicus* larvae. Some reports have shown that yeast *S. cerevisiae* has been recognized to have potential as a substitute for live food in the production of certain fish or as a potential replacement for fish meal and potential of probiotic (Oliva-Teles and Goncalves, 2001). In experimental trials, the *S. cerevisiae* optimized the feed consumption of *A. persicus* larvae. In the probiotic experimental treatments dry matter, crude lipid and crude energy of *Acipenser persicus* larvae significantly increased and the FCR and RFI with the present of yeast significantly decreased.

The other feeding parameters as PPV, LPV, EPV, PER, EER and FCE SGR, GCE and TGC significantly increased. Similar effects had been reported for other fishes to increase considerably with the use of probiotic in the diet (Tovar-Ramirez *et al*., 2004) and they were indicated that the yeast *S. cerevisiae* increased feeding parameters and better feeding efficiency in sea bass and Tovar *et al*. (2002) reported that when the diet was supplemented with a suitable amount of the yeast, the sea bass (*Dicentrarchus labrax*) larvae grew faster. Suggesting that isolated yeast as *S. cerevisiae* improve the growth in cultivable fish larvae (Andlid *et al*., 1995).

In this study different results of growth parameters were obtained from using different levels of *S. cerevisiae* in enrichment of *A. urmiana*. The growth parameters of FBW, VW and VL in probiotic trials of T1 and T2 had the highest levels in comparison with control.

Noh *et al*. (1994) and Bogut *et al*. (1998) also studied the effect of supplementing carp feeds with different additives including antibiotics, yeast (*S. cerevisiae*) and bacteria, they obtained the best growth with a bacterium not yeast. But their conclusion in carp was in contrast to the results.

Nevertheless, the backer's yeast, *S. cerevisiae* had negative effects on growth parameters of feeding performance when was used in concentration of 5.48 Log CFU mL^{-1} in suspension of broth. The similar

effects of *S. cerevisiae* were reported by Jafaryan *et al.* (2009) in feeding of Raibow Trout (*Oncorhynchus mykis*) larvae when the Trout larvae were fed on supplemented diet by blend of isolated sturgeon gut bacillus and yeast of *S. cerevisiae* in level of 4.30 Log CFU g^{-1} of feed, it had the maximum of growth parameters and survial. While use the over dose (5.30 and 6.30 LogCFU g^{-1} of feed) of yeast, decreased the growth and survival rate of Trout larva. However, in trial T2, the Persian sturgeon larvae were fed by bioencapsulated *A. urmiana* in level of 5.30 Log CFU mL^{-1} of suspension of broth had the best results in growth and feeding parameters.

CONCLUSION

The results of the present experiment highlighted that Persian Sturgeon larvae effectively could with *S. cerevisiae* show high efficiency in exploitation of nutrient compositions, promotion of body compounds, growth performance and survival rate.

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